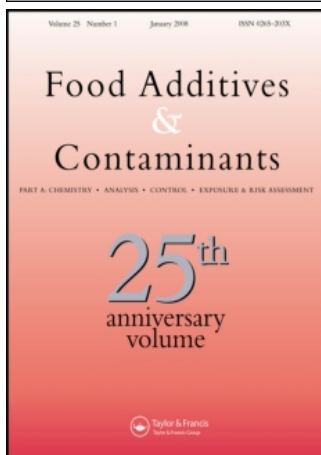


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## Food Additives & Contaminants Part A - Chemistry, Analysis, Control, Exposure & Risk Assessment

Publication details, including instructions for authors and subscription information:  
<http://www.informaworld.com/smpp/title~content=t713599661>

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Online Publication Date: 01 February 2008

To cite this Article: Dorner, J. W. (2008) 'Management and prevention of mycotoxins in peanuts', Food Additives & Contaminants, 25:2, 203 - 208

To link to this article: DOI: 10.1080/02652030701658357

URL: <http://dx.doi.org/10.1080/02652030701658357>

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## Management and prevention of mycotoxins in peanuts

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(Received 31 May 2007; accepted 19 August 2007)

### Abstract

Contamination of peanuts with mycotoxins, particularly aflatoxins, is a worldwide problem that affects both food safety and agricultural economies. Most countries have adopted regulations that limit the quantity of aflatoxins in food and feed to  $20 \mu\text{g kg}^{-1}$  or less; however, environmental conditions in most of the world where peanuts are produced and stored often make it difficult or impossible to attain such low concentrations. In addition to aflatoxins, peanuts are often contaminated with cyclopiazonic acid (CPA). Both mycotoxins are produced by *Aspergillus flavus*, a ubiquitous fungus that can infect and grow in peanuts under both pre- and post-harvest conditions. Management of mycotoxin contamination in peanuts generally involves removal of high-risk components from shelled lots or the removal of individual, highly contaminated nuts. This is accomplished by various processes such as screening, kernel sizing, electronic colour sorting, hand sorting, and blanching followed by electronic colour sorting. Recently, biological control technology has been developed that prevents much of the contamination that might otherwise occur. Biocontrol is based on competitive exclusion whereby a dominant population of a non-toxigenic strain of *A. flavus* is established in the soil before peanuts are subjected to conditions favouring contamination. The applied strain competes with toxigenic strains for infection sites, resulting in significantly reduced concentrations of aflatoxins in peanuts. Monitoring of the first commercial use of the technology showed that aflatoxins were reduced by an average of 85% in farmers' stock peanuts and by as much as 98% in shelled, edible grade peanuts.

**Keywords:** Peanut, aflatoxin, cyclopiazonic acid, *Aspergillus flavus*, *Aspergillus parasiticus*, segregation, electronic colour sorting, biological control, prevention, management

### Introduction

The birth of mycotoxicology came about with the discovery of the aflatoxins in the early 1960s (Sargeant et al. 1961), and because they were found as contaminants of Brazilian peanut meal, there has been long-standing concern about the safety of peanuts as food and feed. Aflatoxins are produced in peanuts as a result of invasion and growth by *Aspergillus flavus* and *A. parasiticus*, and contamination can occur during various stages of production, harvest, handling, and storage (Diener et al. 1987). Pre-harvest aflatoxin contamination of peanuts is associated with late-season drought conditions as peanuts begin to dehydrate in the soil under hot, dry environmental conditions (Cole et al. 1989). Contamination can also occur after peanuts are dug if they are not quickly harvested, dried, and

maintained at a safe moisture level. In addition to aflatoxins, peanuts can be contaminated with cyclopiazonic acid (CPA), another mycotoxin produced by *A. flavus* as well as other species of *Aspergillus* and *Penicillium* (Lansden & Davidson 1983). Although not as acutely toxic as aflatoxins (the oral  $\text{LD}_{50}$  in rodents is approximately  $30\text{--}70 \text{ mg kg}^{-1}$ ), CPA is a potent inhibitor of the reticular form of the  $\text{Ca}^{2+}$ -ATPase pump. A thorough review of the toxicology and a safety assessment of CPA has been published recently (Burdock & Flamm 2000). CPA has been found as a natural contaminant of a variety of commodities and foods, and it was implicated in a human poisoning associated with kodo millet (Rao & Husain 1985). The vast majority of CPA contamination of peanuts likely results from *A. flavus* and quite often co-occurs with aflatoxins (Urano et al. 1992). Because peanuts are primarily produced in

tropical and subtropical-to-temperate regions, *A. flavus* and *A. parasiticus* are the predominant mycotoxigenic fungi associated with peanuts, and significant contamination of peanuts with other mycotoxins is rare. Therefore, the focus of this paper will be on measures to manage and prevent aflatoxin contamination with the understanding that these same measures are also effective for the management and prevention of CPA contamination.

Although aflatoxins are potent hepatotoxins, concern about their potent carcinogenicity has forced government regulatory agencies to establish very low tolerances for aflatoxins in food, including peanuts and peanut products (Van Egmond 2002). The European Union upper limit for aflatoxins in peanuts is  $2 \mu\text{g kg}^{-1}$  for aflatoxin  $B_1$  and  $4 \mu\text{g kg}^{-1}$  for total aflatoxins ( $B_1 + B_2 + G_1 + G_2$ ) (European Commission 1998). The upper limit set by the US Food and Drug Administration is  $20 \mu\text{g kg}^{-1}$  for total aflatoxins (<http://www.cfsan.fda.gov/~lrd/fdaact.html>), but the US peanut industry maintains a self-imposed limit of  $15 \mu\text{g kg}^{-1}$  that is administered by the US Department of Agriculture (USDA) (Whitaker et al. 2005). Most other countries have adopted similar regulations (Food and Agriculture Organization 2004). Because individual peanut seeds can be contaminated with aflatoxin concentrations as high as several hundred thousand to a million  $\mu\text{g kg}^{-1}$  coupled with the fact that usually very few seeds are contaminated, sampling error makes it very difficult to ensure that shelled lots meet the low regulatory limits (Whitaker et al. 1974). The scientific research community, in conjunction with peanut industries, have worked very hard to ensure that edible-grade peanuts contain the lowest aflatoxin concentrations possible. However, it is not always possible to achieve the necessary level, and this produces severe economic pressure on commercial peanut companies during years when aflatoxin contamination is severe. The purpose of this paper is to review the techniques that have been developed for managing aflatoxin contamination when it occurs and also to describe a newly developed methodology for preventing much of that contamination.

### Management techniques

Aflatoxin management techniques are those that have been developed to manage contamination that has already occurred. That contamination could have taken place during any of several phases in the production of edible-grade peanuts, including: (1) in the field under late-season conditions of drought and heat stress; (2) after peanuts were dug, but before they could be harvested, usually a result of rainy conditions after digging; (3) during transport of

peanuts from the field to the point of sale when there could be delays in drying; and (4) during storage of farmers' stock (FS) or shipment of shelled peanuts when a safe storage moisture content cannot be maintained. Most of the techniques used to manage this contamination involve the physical separation of contaminated from uncontaminated seed.

### Lot segregation

In the USA, the first opportunity to manage aflatoxin contamination occurs when FS lots are delivered by the farmer to the point of sale (buying point). As part of the official grading procedures performed on all FS lots, a sample of peanuts taken from the lot is visually inspected for the presence of aflatoxin-producing fungi. If a peanut is found with visible *A. flavus* or *A. parasiticus*, the entire lot is diverted from the edible supply and can be used only for oil (Whitaker et al. 1998). Although no 'official' aflatoxin analysis is conducted on incoming FS peanuts, many buyers perform independent analyses on lots in which visible *A. flavus* is not found to determine better the aflatoxin risk associated with incoming lots. Buyers can then segregate lots for storage based on the concentration of aflatoxin found. Management systems in place in other peanut-producing countries vary, and some include an aflatoxin analysis on incoming FS peanuts with price deducts based on the amount of aflatoxin. After a period of storage, during which time aflatoxin may be produced or increase, peanuts are subjected to some or all of the management techniques described below to produce shelled lots that meet specific regulatory guidelines.

### Screening

After lot segregation the first step in managing aflatoxin contamination usually involves running peanuts over a screening device to separate certain high-aflatoxin-risk components. High levels of aflatoxin have been shown to be associated with loose shelled kernels (LSK), which are peanuts that have been dislodged from their pods during the harvesting and handling processes (Dorner & Cole 1997). In addition, higher levels of aflatoxin are associated with small, immature pods. Therefore, removing these high-risk components before storing or shelling has the effect of reducing the aflatoxin concentrations subsequently found in shelled lots. For many years these separations were accomplished with vibratory, perforated screens that were not widely utilized because of low peanut flow rates and a tendency for perforations to become clogged. However, development of the 'belt screen' has greatly increased the screening of FS peanuts before shelling. The belt screen is a series of parallel

belts spaced apart at specific distances which rotate continuously around appropriately positioned sheaves. As peanuts flow across the rotating belts, materials smaller than the spaces between the belts (such as LSK and small pods) fall through while larger pods ride across to the end for collection (Smith et al. 1995). The belts can be spaced to allow for very efficient separation of LSK, small pods, and some foreign material resulting in reduced aflatoxin in final shelled product. In a study of the effect of belt screening 17 loads of FS peanuts, Dowell et al. (1990) found an average 35% reduction in aflatoxin as a result of screening. These devices are now widely used in the USA peanut industry.

#### *Density segregation*

After peanuts are shelled, they are fed to a gravity table that separates material based on specific gravity. This is done primarily to remove foreign material as well as to separate unshelled pods from the shelled kernels. Because highly contaminated kernels are less dense than most kernels, a reduction in aflatoxin contamination of the final product can be achieved by separately collecting the least dense kernels from the gravity table (Davidson et al. 1981). From an initial average FS lot concentration of  $60 \mu\text{g kg}^{-1}$ , Davidson et al. found average aflatoxin concentrations of 10.2, 44.5, and  $69.6 \mu\text{g kg}^{-1}$  in the heavy, medium, and light fractions, respectively, after density segregation. Use of this methodology for the specific purpose of managing aflatoxin is not a matter of routine practice because of a lack of efficiency. Too many non-contaminated kernels are lost in the light fraction to make it economical. However, during crop years characterized by unusually high levels of aflatoxin separation of the very lightest kernels from the main flow can remove enough aflatoxin to make the process worthwhile.

#### *Kernel sizing*

After peanuts are shelled, kernels are separated into different size categories by passing peanuts over a series of slotted and round-hole screens (Whitaker et al. 2005). Edible grade runner-type peanuts in the USA are classified as jumbo (kernels that ride a screen with 0.833 cm wide by 1.9 cm long slotted holes [21S]), medium (kernels that fall through the 21S screen but ride a screen with 0.714 cm wide by 1.9 cm long slotted holes [18S]), number one (kernels that fall through the 18S screen but ride a screen with 0.675 cm diameter round holes [17R]), and sound splits (kernels that split during shelling and ride a 17R screen). Kernels that fall through the 17R screen are classified as oil stock and are not used for edible purposes. Although shelled peanuts are not sized for the purpose of managing aflatoxin

contamination, that is a by-product of the process because higher concentrations of aflatoxin are associated with smaller size kernels. This association is actually based on the maturity of peanut pods, with immature pods being more susceptible to contamination than mature pods (Dorner et al. 1989). Generally, immature pods contain smaller kernels than mature pods. Therefore, much of the aflatoxin in farmers' stock peanuts is found in immature kernels that end up in the oil stock category. As the size of kernels increases, generally lower concentrations of aflatoxin are found. The jumbo and medium size categories can account for about 70% of the total weight of kernels in farmers' stock peanuts. In a study of the partitioning of aflatoxin into various size categories using a 45 kg sample from 46 farmers' stock lots, Whitaker et al. (2005) found that the initial mean aflatoxin concentration of  $73.7 \mu\text{g kg}^{-1}$  was reduced to means of 42.5 and  $66.2 \mu\text{g kg}^{-1}$  in the jumbo and medium size categories, respectively, but was increased to 93.6, 105.1, and  $133.6 \mu\text{g kg}^{-1}$  in the number one, sound split, and oil stock categories, respectively.

#### *Electronic colour sorting*

The most effective technique for managing aflatoxin contamination in commercial shelling plants is electronic colour sorting (ECS). In shelling plants in the USA all peanuts pass through these high-speed sorters to remove discoloured kernels. This is done to improve overall quality, including the reduction of aflatoxin in the final product. Peanuts that have been colonized by aflatoxigenic fungi are often discoloured, and ECS very efficiently removes a high percentage of the contaminated, discoloured kernels. In a study that evaluated the effect of various post-harvest aflatoxin management techniques, ECS produced a 70% reduction in the amount of aflatoxin in the medium kernel size category (Cole et al. 1995). In recent years continued advances in ECS technology have improved sorter efficiency with the result that fewer 'good' kernels are rejected. However, not all aflatoxin-contaminated kernels are discoloured; therefore, ECS is never 100% effective in aflatoxin removal.

#### *Blanching and electronic colour sorting*

The final technique that can be employed to reduce the aflatoxin concentration in shelled peanut lots is blanching followed by ECS. Blanching is a procedure that removes the testa (seed coat) from kernels. ECS after blanching very efficiently removes aflatoxin-contaminated kernels from the blanched lot because slight discolorations in the kernel tissue that were not visible before testa removal become evident after removal. Because aflatoxin contamination is

often associated with these discolorations, blanching followed by ECS is widely recognized as the best method for reducing aflatoxin in shelled peanut lots, and it is usually performed at a facility specifically designed for this process. When shelled lots are found to contain aflatoxin concentrations above prescribed limits, thus precluding their sale, they are often sent to a blanching facility in order to reduce the concentration to an acceptable level. In the study reported by Cole et al. (1995), blanching/ECS produced a 91% reduction in the mean aflatoxin concentration of a lot of shelled medium peanuts. The major disadvantage to this form of aflatoxin reduction is the cost, which includes US\$0.075/lb in direct charges, the weight loss incurred during blanching, and the loss of kernels by ECS (Dorner & Lamb 2006).

### Prevention techniques

The best way to control mycotoxin contamination of peanuts is to prevent it in the first place. This is not always possible, but technologies exist which, if available and affordable, can prevent much of the contamination that would otherwise occur.

#### *Kernel moisture control*

Pre-harvest aflatoxin contamination of peanuts essentially can be eliminated with proper and adequate irrigation. Developing and maturing peanuts are not susceptible to colonization by *A. flavus* and *A. parasiticus* until kernel moisture (water activity) begins to decrease in response to late-season drought conditions with increased soil temperature (Dorner et al. 1989). Maintaining high kernel water activity until the time of harvest maintains the natural defence mechanism (phytoalexin production) of peanuts against growth by aflatoxigenic fungi, even if fungal invasion occurs. The only exception to this is under severe insect pressure whereby extensive pod damage may give the fungi the opportunity to overwhelm the ability of kernels to ward off the fungal attack. Unfortunately, many peanut farmers do not have access to supplemental irrigation or the cost is not affordable. After peanuts are dug and harvested, contamination can be prevented by rapidly drying peanuts to or below a water activity (0.83) that cannot support aflatoxin production (Diener & Davis 1970). It is then necessary to maintain that safe storage moisture until peanuts are processed. This can also be difficult or impossible to accomplish because of environmental conditions during harvest as well as during the storage period. Nevertheless, control of kernel moisture is the best way to prevent mycotoxin contamination of peanuts if the means are available.

#### *Aflatoxin risk forecasting for early harvesting*

Pre-harvest aflatoxin contamination of peanuts can be prevented if peanuts are harvested before aflatoxin is actually produced. The time under late-season drought conditions that is necessary for aflatoxin contamination to occur varies and is dependent on numerous factors, the most important being soil temperature. Data from several years of studies conducted at the USA National Peanut Research Laboratory were used to develop aflatoxin prediction models that could be used to forecast when aflatoxin contamination was likely to occur in farmers' fields (Thai et al. 1990; Parmar et al. 1997). Farmers could then use that information to include aflatoxin risk in making harvest decisions. However, that technology never has been seriously utilized in the USA because the marketing system for FS peanuts does not allow for economic penalties based on a measure of aflatoxin. Rather, farmers are penalized if incoming loads are found to contain visible *A. flavus*, but such a finding is relatively rare except under the harshest of conditions. Therefore, harvest decisions are still primarily made to achieve the highest yield possible.

In Australia, however, where penalties are imposed based on the quantity of aflatoxin found in FS loads, a web-based aflatoxin risk-prediction system called AFLOMAN (<http://www.apsim.info/apsim/afloman/>) has recently been employed (Wright et al. 2005). Farmers input information on daily rainfall and soil and ambient temperatures via the internet. The Agricultural Production Systems Simulator (APSIM) peanut aflatoxin model is then run for the specific field with results uploaded back to the website. Farmers can view graphs showing changes in the fraction of available soil water, soil temperature, and aflatoxin risk. The risk of aflatoxin contamination can then be taken into account so that earlier-than-normal harvesting can be undertaken to minimize aflatoxin contamination.

#### *Biological control*

New biological control technology has been developed that can prevent much of the contamination of peanuts with aflatoxins and CPA that would otherwise occur. That control is based on competitive exclusion and is achieved by applying a competitive, non-toxicogenic strain of *A. flavus* to the soil of developing peanuts. Most of the research that resulted in development of this technique has been recently reviewed (Dorner 2005). It was shown that biological control is effective for both pre- and post-harvest aflatoxin contamination. The technology has been commercialized and the biocontrol product afla-guard® has been registered by the US Environmental Protection Agency (2004) as

a biopesticide for control of aflatoxin contamination in peanuts.

The biopesticide is hulled barley that is coated with conidia of a non-toxigenic strain of *A. flavus* (NRRL 21882). The strain is not only a non-producer of aflatoxins, but also it does not produce CPA or other aflatoxin biosynthetic precursors (Dorner 2004). Genetic analysis of the strain revealed a deletion of the entire aflatoxin gene cluster (Chang et al. 2005). Ideally, afla-guard is applied to the peanut crop at 60–80 days after planting, or soon after canopy closure. After application and uptake of moisture the coated conidia germinate and grow, producing abundant sporulation that is disseminated into the soil for competition with toxigenic strains that are naturally present. Research studies have shown that this biological control strategy can produce reductions in aflatoxin contamination of approximately 80–90% (Dorner et al. 1998; Dorner 2004).

In 2004, studies were carried out to monitor the efficacy of commercial applications of afla-guard (Dorner & Lamb 2006). FS peanuts treated with afla-guard from seven locations in Georgia and Alabama in the USA were found to have an overall mean reduction in aflatoxin of 85.2%. At two locations where treated and untreated peanuts were stored for several months and then shelled, mean aflatoxin reductions in edible grade peanuts were 69 and 98%, respectively. At both locations no shelled lots of treated peanuts tested above the USDA limit of  $15 \mu\text{g kg}^{-1}$  compared with 15.8 and 48.4% of untreated peanuts at the respective locations. Economic analysis based on the costs associated with the blanching of failed lots showed that the use of the biopesticide produced a net increase in shelled stock value at the two locations of 6.1 and 15.3%, respectively.

## Conclusions

Mycotoxin contamination of peanuts, which can occur both before and after harvest, can be effectively managed to produce shelled peanuts that meet strict regulatory guidelines, ensuring a safe food supply. This management primarily involves techniques that remove highly contaminated kernels from the majority that are not contaminated. However, these removal steps are costly, in terms of both processing and unavoidable loss of non-contaminated kernels. It is highly preferable to take steps to prevent contamination if at all possible. Such steps include control of kernel moisture both before and after harvest. If that is not possible, prevention of pre-harvest aflatoxin contamination can be achieved by harvesting peanuts

before contamination occurs. However, this early harvesting can result in reduced yield and reduced income for the farmer. Biological control technology has recently been commercialized which prevents much of the contamination that would otherwise occur. Best practices to achieve the lowest possible levels of contamination are to combine all possible management and prevention strategies to ensure and maintain a safe supply of peanuts.

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